

Abietic acid activates peroxisome proliferator-activated receptor- γ (PPAR γ) in RAW264.7 macrophages and 3T3-L1 adipocytes to regulate gene expression involved in inflammation and lipid metabolism

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Abstract Abietic acid is one of the terpenoids, which are multi-functional natural compounds. It has been reported that abietic acid suppresses effects on inflammation. However, the mechanism underlying the anti-inflammatory effects remains unclear. The present work indicates that abietic acid suppresses the protein expression of tumor necrosis factor- α and cyclooxygenase 2, which are involved in inflammation, in lipopolysaccharide-stimulated macrophages. Moreover, this effect resembles that of thiazolidinedione, a synthetic peroxisome proliferator-activated receptor- γ (PPAR γ) ligand. Indeed, abietic acid activates PPAR γ in luciferase reporter assays. The activity of abietic acid induces PPAR γ target gene expression in RAW264.7 macrophages and 3T3-L1 adipocytes. These data indicate that abietic acid is a PPAR γ ligand and that its anti-inflammatory effect is partly due to the activation of PPAR γ in stimulated macrophages. The present work suggests a novel possibility that abietic acid, a naturally occurring compound, can be used not only for anti-inflammation but also for regulating lipid metabolism and atherosclerosis.

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Key words: Abietic acid; Anti-inflammatory; Peroxisome proliferator-activated receptor- γ ; Adipocyte; Macrophage; Terpenoid; Nutrigenomics

1. Introduction

Terpenoids, which are present in many dietary and herbal plants [1], exhibit many biological effects: for example, anti-tumor proliferation, antihypercholesterolemia and antidiabetic

[1–3]. Therefore, terpenoids have been regarded as valuable medicinal and food components that control many cellular functions and medical conditions in vivo.

Abietic acid, a diterpene, is a major component of the rosin fraction of oleoresin synthesized by conifer species, such as grand fir (*Abies grandis*) and lodgepole pine (*Pinus contorta*) [4]. The chemical structure of abietic acid is shown in Fig. 1A. It was reported that abietic acid has an anti-inflammatory effect [5–7]. In lipopolysaccharide (LPS)-stimulated macrophages, abietic acid suppresses production of prostaglandin E₂ (PGE₂) in vitro and in vivo [7]. However, the mechanism underlying the anti-inflammatory effect of abietic acid has not been clarified.

The anti-inflammatory effect is regulated by many transcriptional factors in vivo. Among the transcriptional factors, peroxisome proliferator-activated receptor- γ (PPAR γ), a member of the nuclear receptor superfamily activated by ligands, plays an important role in inflammation [8,9]. The activation of PPAR γ signaling enhances macrophage differentiation, inducing the expression of fatty acid translocase (FAT)/CD36, one of the cell's 'scavenger' receptors for the atherogenic low-density lipoprotein (LDL) [10]. Lipoprotein lipase (LPL) also acts as a bridge, which enhances binding to the extracellular matrix and uptake of both native and oxidized LDL by macrophages. These molecules expressed in macrophages may exhibit pro-atherogenic activities. However, thiazolidinedione (TZD), a synthetic ligand for PPAR γ , suppresses the production of pro-inflammatory cytokines including tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in LPS- or phorbol 12-myristate-13-acetate-stimulated macrophages [11,12]. Recent studies showed that, in addition to the anti-inflammatory effect, TZD regulates expression of genes involved in lipid metabolism in macrophages and suppresses their transformation into foam cells [11,13]. Therefore, regulation of PPAR γ by TZD in macrophages is indispensable not only for anti-inflammatory events but also for the transformation into foam cells. On the other hand, TZD has been widely used as an antidiabetic drug, which activates PPAR γ resulting promotion of adipocyte differentiation ([14], review). TZD not only stimulates glucose uptake into differentiated adipocytes but also induces production of adiponectin (an insulin sensi-

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; TZD, thiazolidinedione; BADGE, bisphenol A diglycidyl ether; LPS, lipopolysaccharide; CREB, cAMP response element-binding protein; CBP, CREB-binding protein; aP2, adipocyte fatty acid-binding protein; LPL, lipoprotein lipase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TNF- α , tumor necrosis factor- α ; COX2, cyclooxygenase 2

tivity-promoting factor) and suppression of TNF- α (a insulin resistance-inducing factor) through PPAR γ activation in adipocytes [15–17]. Thus, ligands for PPAR γ such as TZD can show multiple effects including antidiabetes, anti-inflammation, and antiatherosclerosis.

In this study, we show similarity between abietic acid and TZD in terms of suppressive activity for TNF- α and cyclooxygenase 2 (COX2) induction at the protein level in LPS-stimulated macrophages. Moreover, the results demonstrate that abietic acid activates PPAR γ in the GAL4 chimera system. Indeed, abietic acid could induce the expression of PPAR γ target genes in RAW264.7 macrophages and 3T3-L1 adipocytes. Moreover, the induction of PPAR γ target gene expression depended on the expression of PPAR γ and a nuclear receptor coactivator, cAMP response element-binding protein (CREB)-binding protein (CBP), in NIH3T3 fibroblasts. These data indicate that abietic acid is an activator of PPAR γ and the anti-inflammatory effect of abietic acid is, at least partly, due to its activity as an activator of PPAR γ . The results further suggest that abietic acid is a valuable medicinal and food component for use in control functions of macrophages and adipocytes.

2. Materials and methods

2.1. Chemical reagents

T174 TZD, a specific ligand for PPAR γ [18], was kindly provided by Tanabe Seiyaku (Osaka, Japan). Abietic acid and bisphenol A diglycidyl ether (BADGE) were from Sigma (St. Louis, MO, USA) or Tokyo Chemical Industry (Tokyo, Japan). These compounds were diluted with dimethyl sulfoxide (DMSO) to prepare the stock solutions (10–100 mM). All other chemicals were from Sigma or Nacalai Tesque (Kyoto, Japan) and of guaranteed reagent grade or tissue culture grade.

2.2. Cell culture

Monkey CV1 kidney cells, mouse 3T3-L1 preadipocytes, mouse NIH3T3 fibroblasts and mouse RAW264.7 macrophage were purchased from American Type Culture Collection. They were cultured in a maintenance medium (10% fetal bovine serum, 200 μ M ascorbic acid, 100 units/ml penicillin and 100 μ g/ml streptomycin in Dulbecco's modified Eagle's medium) at 37°C in 5% CO₂/95% air under humidified condition. For adipocyte differentiation, 3T3-L1 preadipocytes were incubated in a differentiation medium 2 days after they reached confluence, as described previously [19]. Cell viability was measured using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay as previously described [20].

2.3. Preparation of peritoneal macrophages

BALB/c male mice (Japan SLC, Hamamatsu, Japan) were injected intraperitoneally with 3–4 ml of thioglycollate broth (Difco, Detroit, MI, USA) (3%) 4 days before use. Peritoneal macrophages were prepared as follows: lightly ether-anesthetized mice ($n=4-6$) were injected intraperitoneally with 5 ml of sterilized phosphate-buffered saline. The peritoneal fluid was carefully aspirated to avoid hemorrhage. After centrifugation at 200 \times g for 10 min, the cell pellet was washed twice with the maintenance medium. Cells were seeded at 1×10^6 /ml in the maintenance medium. After incubation for 2 h at 37°C, non-adherent cells were removed by extensive washing with the maintenance medium. All animals received human care, as outlined in the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee according to NIH 86-23; revised 1985).

2.4. Measurement of TNF- α and Western blot analysis of COX2

The concentration of TNF- α in culture supernatants was assayed by enzyme-linked immunosorbent assay (ELISA). The assays were conducted utilizing OptEIA™ Mouse TNF- α set (Pharmingen, San Diego, CA, USA). The samples were thawed, diluted properly in assay diluents, and thoroughly mixed. Samples (triplicate) and stan-

dards (duplicate) were pipetted into the appropriate wells of the TNF- α antibody-coated ELISA plate. Plates were sealed and incubated for 2 h at room temperature. After rinsing thoroughly, each well was incubated for 1 h with 100 μ l of biotinylated mouse TNF- α monoclonal antibody and streptavidin–horseradish peroxidase conjugate. Following another rinse, tetramethylbenzidine substrate solution was placed in each well in the dark for 30 min. The absorbance of each well was read at 450 nm on a microplate reader. Cytokines were quantitated from standard curves using the SOFTmax curve-fitting program (Molecular Devices, Sunnyvale, CA, USA).

For Western blotting to analyze the expression of COX2, mouse monoclonal COX2 antibody (Transduction Labs, Lexington, KY, USA) was used. Western blotting was performed as previously described [20].

2.5. Luciferase assays

Our luciferase assays were performed using an advanced highly sensitive system with coexpression of coactivator CBP developed by modifying the dual luciferase system (Promega, Madison, WI, USA), as previously described [21]. Twenty-four hours after transfection of both reporter and internal control plasmids, the transfected cells were cultured in the medium containing each compound for another 24 h. Luciferase assays were performed using the dual luciferase system according to the manufacturer's protocol.

2.6. RNA preparation and quantification of gene expressions

RNA samples of differentiated 3T3-L1 were prepared using cells cultured on 6-well tissue culture plates 8 days after differentiation induction. The cells were treated with the medium containing PPAR γ ligand for 48 h before RNA sample preparation. For analysis of gene expression in RAW264.7 and NIH3T3, pSG5-mPPAR γ , the expression vector for PPAR γ , was transfected into RAW264.7 cultured on 6-well plates with LipofectAMINE and Plus reagents (Invitrogen, San Diego, CA, USA) as previously described [21]. Twenty-four hours after the transfection, the transfected RAW264.7 and NIH3T3 cells were treated for another 24 h with the experimental medium containing each sample compound. All the total RNA samples were prepared from cells using the SV total RNA isolation system (Promega) according to the manufacturer's protocol.

Aliquots of total RNA were reverse-transcribed using M-MLV reverse transcriptase (Invitrogen) in a thermal cycler (Takara PCR Thermal Cycler SP; Takara Shuzo, Kyoto, Japan) according to the manufacturer's instructions. To quantify mRNA expression, polymerase chain reaction (PCR) was performed using a quantitative real-time RT-PCR system (LightCycler System; Roche Diagnostics, Mannheim, Germany), as described previously [19,21]. All primer sets used in this study were described previously [21]. To compare the mRNA expression levels among samples, the copy numbers of each transcript were divided by that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) showing a constant expression level.

2.7. Statistical analysis

The data are presented as means \pm S.E.M. and were statistically analyzed using the unpaired *t*-test or the Welch *t*-test when variances were heterogeneous. Differences were considered significant when *P* was <0.05 .

3. Results

3.1. Abietic acid and PPAR γ ligand suppress expression of TNF- α and COX2 in LPS-stimulated macrophages

To investigate the anti-inflammatory effect of abietic acid, LPS-stimulated peritoneal macrophages were cultured in the medium containing abietic acid. The addition of 50 μ M abietic acid suppressed LPS-induced production of TNF- α (16.3% of the LPS-stimulated vehicle control) as shown in Fig. 1B. This suppressive effect of abietic acid on TNF- α production was dose-dependent. Under the same experimental conditions, 5 μ M TZD, which regulates the expression of macrophage differentiation marker genes and inflammation-related genes via PPAR γ activation, suppressed approximately 8.9% of the LPS-induced TNF- α production. Moreover, the

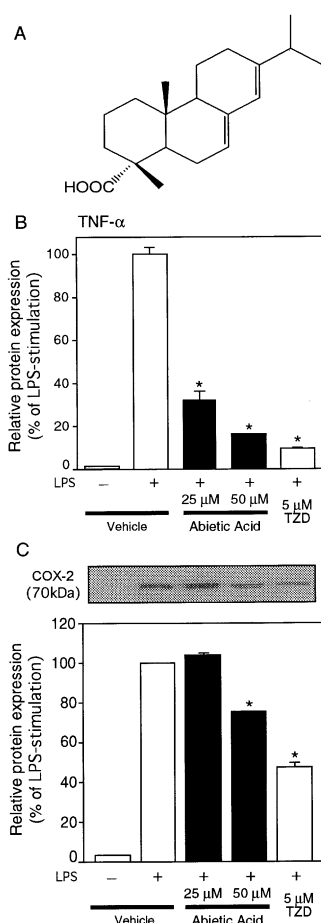


Fig. 1. Abietic acid suppresses protein expression of pro-inflammatory markers in LPS-stimulated macrophages. Structure of abietic acid (A). Peritoneal macrophages were stimulated with 25 ng/ml LPS and incubated for 24 h with abietic acid or TZD. B: Suppressive effects of abietic acid and TZD in LPS-induced TNF- α production. The quantification of TNF- α released from LPS-stimulated macrophages was performed using ELISA as described in Section 2. The production level of LPS-induced TNF- α was set at 100%. The values are means \pm S.E.M. of four tests. * P < 0.05 compared with LPS-induced TNF- α production. C: Suppressive effects of abietic acid and TZD in LPS-induced COX2 production. Equal amounts of protein (15 μ g/lane) were loaded and electrophoresed on a 10% sodium dodecyl sulfate–polyacrylamide gel. After the protein separation, proteins were blotted onto a nitrocellulose membrane, and then COX2 was detected using a chemiluminescent system (ECL). The data are representative of three independent blots. The values are means \pm S.E.M. of three tests. * P < 0.05 compared with LPS-induced COX2 expression.

protein expression of COX2, which is enhanced in LPS-stimulated macrophages, was suppressed to 75.6% and 45.3% of the LPS-stimulated vehicle control by 50 μ M abietic acid and 5 μ M TZD, respectively (Fig. 1C), although addition of a lower concentration of abietic acid (25 μ M) had no effect. These suppressive effects of abietic acid on the protein expression of TNF- α and COX2 were confirmed at the mRNA level by quantification of both mRNAs in stimulated peritoneal macrophages by using quantitative RT-PCR (the addition of 50 μ M abietic acid resulted in 65% and 80% of the vehicle controls for the mRNA expressions of TNF- α and COX2, respectively). These results suggest that abietic acid suppresses the protein expression of both TNF- α and COX2 and that the

suppressive effects of abietic acid and TZD are governed by the same mechanism.

3.2. Abietic acid activates PPAR γ as determined by luciferase assays

We hypothesized that PPAR γ is activated by abietic acid as well as TZD, because of the similarity between abietic acid and TZD in terms of their anti-inflammatory effects. Thus, we carried out reporter assays for PPAR γ ligands using abietic acid. In the assays, abietic acid induced GAL4/PPAR γ chimera transactivation in a dose-dependent manner (Fig. 2A). Using the GAL4/PPAR γ chimera system, 30 μ M abietic acid induced activation 4.6-fold that of the vehicle control (Fig. 2A). The concentrations of abietic acid used in these assays had no influence on cell viability (data not shown). Moreover, in the GAL4/PPAR γ chimera system, BADGE, a PPAR γ antagonist [22], suppressed luciferase activity of 10 μ M of abietic acid in a dose-dependent manner (Fig. 2B). The activity of abietic acid in the presence of 1 μ M BADGE was significantly suppressed at 41% of the activity in the absence of BADGE. It was shown that a binding site of this compound in the ligand-binding domain (LBD) of PPAR γ is the same as that of TZD, as determined by competitive assays using 3 H-labeled TZD [22]. Therefore, these results indicate that abietic acid activates PPAR γ as determined by luciferase assays using the GAL4/PPAR γ chimera system and suggest that abietic acid, as well as TZD, binds to the LBD of PPAR γ .

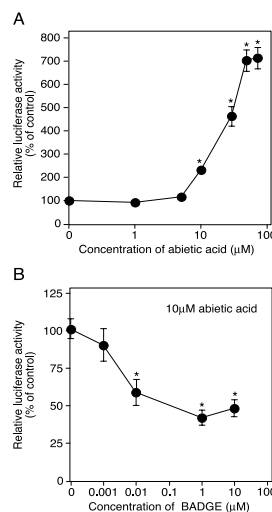


Fig. 2. Abietic acid activates PPAR γ as its ligand. A: Luciferase ligand assays using the PPAR γ /GAL4 chimera system in CV1 cells. pM-hPPAR γ , p4xUASg-tk-luc, pCMX-CBP and pRL-CMV (an internal control) were transfected into CV1 cells and then, 24 h after the transfection, the cells were treated with abietic acid at various concentrations for 24 h. The activity of the vehicle control was set at 100% and the relative luciferase activities are presented as fold induction to that of the vehicle control. The values are means \pm S.E.M. of four tests. * P < 0.05 compared with the vehicle control. B: Antagonization of activity of abietic acid as a PPAR γ ligand by BADGE. The same plasmids as used in ligand assays above were transfected into CV1 cells and 24 h after the transfection 10 μ M abietic acid was added into the culture medium with or without BADGE at various concentrations. After another 24 h, luciferase assays were performed. The activity of 10 μ M abietic acid without BADGE was set at 100% and the relative luciferase activities are presented as fold induction to that of the positive control. The values are means \pm S.E.M. of four tests. * P < 0.05 compared with control without BADGE.

3.3. Expression of PPAR γ target genes is induced by abietic acid in macrophages and adipocytes

To confirm whether abietic acid activated PPAR γ in macrophages, we investigated the mRNA expression of PPAR γ target genes in RAW264.7. In transiently PPAR γ -expressing RAW264.7 (the transfection efficiencies were approximately 40% through all experiments), the expression of *FAT/CD36* was induced 2.1-fold following the addition of 50 μ M abietic acid (closed columns in Fig. 3A), whereas that of *LPL* was induced 4.9-fold (open columns in Fig. 3A). Mock control cells showed no induction after addition of each compound (data not shown). The expressions of *FAT/CD36* and *LPL* are controlled by PPAR γ in macrophages [23,24]. Therefore, these results suggest that abietic acid activates PPAR γ to regulate expression of PPAR γ target genes in RAW264.7 macrophages.

Next, to examine the effects of abietic acid on other cells, we quantified the induction of PPAR γ target gene expression in 3T3-L1 adipocytes by abietic acid. In differentiated 3T3-L1 adipocytes, 25 μ M abietic acid induced 8.1-fold adipocyte fatty acid-binding protein (aP2) expression 7 days after differentiation induction (closed columns in Fig. 3B). In the case of *LPL*, expression was induced 5.1-fold following the addition of 25 μ M abietic acid (open columns in Fig. 3B). Moreover, these effects of abietic acid were dose-dependent, because addition of 50 μ M abietic acid resulted in 11.4- and 5.8-fold increases in the expression levels of aP2 and *LPL*, respectively. This suggests that abietic acid can induce expression of aP2 and *LPL* via PPAR γ activation in adipocytes.

3.4. The PPAR γ target gene induction by abietic acid depends on expression of PPAR γ and coactivator

To elucidate the details of PPAR γ target gene induction by abietic acid, we used transiently PPAR γ -expressing NIH3T3 fibroblasts (the efficiency of transient transfection was approximately 55% through all experiments), which differentiate into adipocyte-like cells in a PPAR γ -ligand-dependent manner [25]. Addition of 25 μ M abietic acid induced the expression of aP2 7.2-fold in the transiently PPAR γ -expressing NIH3T3 as shown in Fig. 4A. The effect of abietic acid was dose-dependent (50 μ M abietic acid induced 32-fold expression). However, induction of aP2 expression was not observed in the

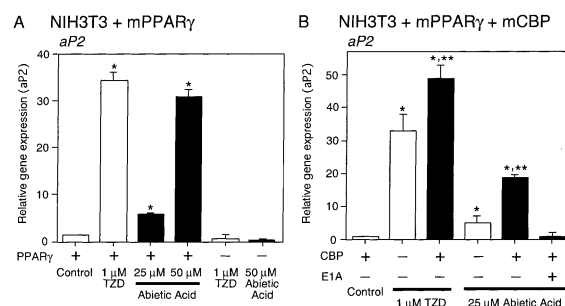


Fig. 4. Abietic acid regulates expression of PPAR γ target genes in NIH3T3 cells. A: NIH3T3 cells transfected with or without a PPAR γ expression vector (pSG5-PPAR γ , 1.0 μ g/well) were cultured on 6-well plates and 24 h after transfection TZD or abietic acid was added to the culture medium and further incubated for 48 h. B: CBP expression vector (pCMX-CBP, 1.0 μ g/well) with PPAR γ expression vector (pSG5-PPAR γ , 1.0 μ g/well) was transiently introduced into NIH3T3 cells cultured on 6-well plates. Twenty-four hours after the transfection, 25 or 50 μ M abietic acid was added into the medium and further incubated for another 24 h. Adenovirus oncoprotein E1A expression vector (1.0 μ g/well)-transfected cells were used as the negative control. In both experiments, the expression level of mRNAs was estimated using LightCycler and normalized to the *GAPDH* expression level. The relative expression levels are presented as fold induction to that of the vehicle control. All values are means \pm S.E.M. of six tests. *** P < 0.05 compared with vehicle controls and samples without CBP expression, respectively.

absence of PPAR γ expression (Fig. 4A). Similar results were obtained in the induction of *LPL* (data not shown). Mock control cells showed no induction after addition of each compound (data not shown). This strongly suggests that the regulation of PPAR γ target gene expression by abietic acid is due to activation of PPAR γ . In addition to the dependence on PPAR γ expression, the induction of aP2 by abietic acid depended on the expression of CBP, a coactivator for PPAR γ . The coexpression of CBP and PPAR γ resulted in a 2.7-fold induction of aP2 expression in the presence of 25 μ M abietic acid in comparison to the expression of only PPAR γ , as shown in Fig. 4B. Moreover, the enhanced aP2 induction due to the coexpression of CBP and PPAR γ was completely inhibited by adenovirus oncoprotein E1A, which specifically inhibits CBP/p300 functions by its direct binding to the coactivator proteins [26]. This coactivator-dependent activation of abietic acid is the same as that of TZD as previously described [19]. These results suggest that the activation of PPAR γ by abietic acid depends on the expression of a coactivator for PPAR γ , i.e. CBP.

4. Discussion

We showed that abietic acid suppresses the protein expression of TNF- α and COX2 in LPS-stimulated macrophages. It was reported that abietic acid has anti-inflammatory effects such as suppression of PGE₂ production and lipoxigenase activity [6,7]. The expression of COX2 is involved in the production of prostaglandins including PGE₂ in macrophages. Therefore, it is suggested that the suppressive effect of abietic acid on PGE₂ production is partly due to the suppression of COX2 expression in macrophages.

Our present study shows that abietic acid activates PPAR γ in the GAL4 chimera system. This indicates that abietic acid activates PPAR γ as its ligand. The activity of abietic acid as a PPAR γ ligand is strongly supported by the competitive effect

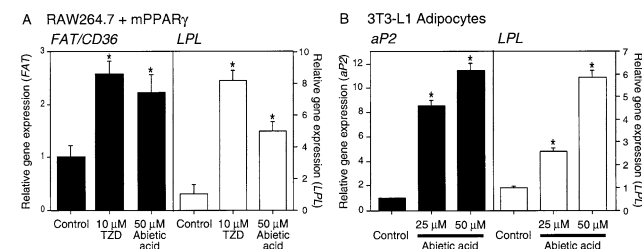


Fig. 3. Abietic acid induces expression of PPAR γ target genes in macrophages and differentiated 3T3-L1 adipocytes. A: Transiently PPAR γ -expressing RAW264.7 cells were cultured on 6-well plates and 24 h after transfection the stimulated cells were treated with or without abietic acid for another 24 h. B: Differentiated 3T3-L1 adipocytes were treated with vehicle control (DMSO), 10 μ M TZD, 25 and 50 μ M abietic acid for 48 h 7 days after differentiation induction. In both experiments, expression of mRNAs was estimated using LightCycler, a quantitative real-time RT-PCR (see Section 2). The relative expression levels are presented as fold induction to that of the vehicle control. All values are means \pm S.E.M. of six tests. * P < 0.05 compared with vehicle controls.

of BADGE (an antagonist of PPAR γ), which binds to PPAR γ LBD, as shown in Fig. 2B. Indeed, abietic acid could regulate the expression of PPAR target genes (*FAT/CD36* and *LPL*) in PPAR γ -expressing RAW264.7 macrophages (Fig. 3A). This induction of PPAR γ target genes by abietic acid depended on PPAR γ expression (Figs. 3A and 4A), suggesting that abietic acid can regulate gene expression via PPAR γ activation as its ligand in intact cells. It has been reported that ligands of PPAR γ such as TZD and 15-deoxy- $\Delta^{12,14}$ -prostaglandin J_2 suppress the expression and/or the production of several inflammatory cytokines including TNF- α , IL-6, IL-2 and PGE $_2$ [9]. This suppressive activity of PPAR γ is due to inhibition of activities of NF- κ B, STAT-1 and AP1 in inflammatory signaling [9]. This is partly because of the competition in binding of CBP/p300 coactivators between PPAR γ and other transcriptional factors [27]. CBP/p300 is essential for ligand-dependent activation of PPAR γ as a coactivator [19]. The PPAR γ activation by abietic acid depended on CBP expression as shown in Fig. 4B. Therefore, it is possible that the anti-inflammatory effects of abietic acid are due to CBP-dependent PPAR γ activation in macrophages.

These results indicate that abietic acid is a functional activator of PPARs in intact cells. It has been reported that other terpenoids including phytanic acid and isoprenols can activate PPARs [20,27]. A common characteristic of such compounds is their activity that regulates the expression of adipocyte differentiation marker genes. Phytanic acid and farnesol increase the expression levels of uncoupling protein-1 in primary cultured brown adipocytes [28], and *aP2/LPL* in 3T3-L1 adipocytes [21], respectively, via PPAR γ activation. In our study, abietic acid upregulated the expression of *aP2* and *LPL* in 3T3-L1 adipocytes. Furthermore, in macrophages it was shown that abietic acid increased the expression levels of *FAT/CD36* and *LPL*, which are associated with lipid metabolism and pathological processes such as atherosclerosis. Therefore, there are possibilities that terpenoids including abietic acid might be classified into a novel class of naturally occurring PPAR ligands and that terpenoids might control lipid metabolism in vivo via PPAR activation. In this sense, it is interesting and might be valuable to analyze the relationship between terpenoids in terms of their structures and abilities to induce PPAR activation.

In conclusion, abietic acid suppresses expression of genes involved in inflammation such as TNF- α and COX2 in activated macrophages. This effect is due to the ability of abietic acid to activate PPAR γ as its ligand. Abietic acid regulates the expression of PPAR γ target genes including *aP2*, *LPL* and *FAT/CD36* in 3T3-L1 adipocytes or RAW264.7 macrophages. In this present work, we propose a possible mechanism of the anti-inflammatory effect of abietic acid and its novel use for transcriptional regulation for management of lipid metabolism in adipocytes or macrophages.

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References

- [1] Elson, C.E., Underbakke, G.L., Hanson, P., Shrago, E., Wainberg, R.H. and Qureshi, A.A. (1989) *Lipids* 24, 677–679.
- [2] He, L., Mo, H., Hadisusilo, S., Qureshi, A.A. and Elson, C.E. (1997) *J. Nutr.* 127, 668–674.
- [3] Miller, A.L. (1998) *Altern. Med. Rev.* 3, 199–207.
- [4] Aranda, F.J. and Villalain, J. (1997) *Biochim. Biophys. Acta* 1327, 171–180.
- [5] Sadhra, S., Foulds, I.S. and Gray, C.N. (1996) *Br. J. Dermatol.* 134, 662–668.
- [6] Ullusu, N.N., Ercil, D., Sakar, M.K. and Tezcan, E.F. (2002) *Phytother. Res.* 16, 88–90.
- [7] Fernandez, M.A., Tornos, M.P., Garcia, M.D., de las Heras, B., Villar, A.M. and Saenz, M.T. (2001) *J. Pharm. Pharmacol.* 53, 867–872.
- [8] Chinetti, G., Fruchart, J.C. and Staels, B. (2000) *Inflamm. Res.* 49, 497–505.
- [9] Glass, C.K. (2001) *J. Endocrinol.* 169, 461–464.
- [10] Lazar, M.A. (2001) *Nat. Med.* 7, 23–24.
- [11] Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J. and Glass, C.K. (1998) *Nature* 391, 79–82.
- [12] Jiang, C., Ting, A.T. and Seed, B. (1998) *Nature* 391, 82–86.
- [13] Rosen, E.D. and Spiegelman, B.M. (2000) *J. Clin. Invest.* 106, 629–631.
- [14] Spiegelman, B.M. (1998) *Diabetes* 47, 507–514.
- [15] Hotamisligil, G.S., Johnson, R.S., Distel, R.J., Ellis, R., Pappaioanou, V.E. and Spiegelman, B.M. (1996) *Science* 274, 1377–1379.
- [16] Maeda, N., Shimomura, I., Kishida, K., Nishizawa, H., Matsuda, M., Nagaretani, H., Furuyama, N., Kondo, H., Takahashi, M., Arita, Y., Komuro, R., Ouchi, N., Kihara, S., Tochino, Y., Okutomi, K., Horie, M., Takeda, S., Aoyama, T., Funahashi, T. and Matsuzawa, Y. (2002) *Nat. Med.* 7, 731–737.
- [17] Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M.L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Tobe, K., Nagai, R., Kimura, S., Tomita, M., Froguel, P. and Kadowaki, T. (2001) *Nat. Med.* 7, 941–946.
- [18] Mizukami, J. and Taniguchi, T. (1997) *Biochem. Biophys. Res. Commun.* 240, 61–64.
- [19] Takahashi, N., Kawada, T., Yamamoto, T., Goto, T., Taimatsu, A., Aoki, N., Kawasaki, H., Taira, K., Yokoyama, K.K., Kamei, Y. and Fushiki, T. (2002) *J. Biol. Chem.* 277, 16906–16912.
- [20] Kim, C.S., Kawada, T., Kim, B.S., Han, I.S., Choe, S.Y., Kurata, T. and Yu, T. (2003) *Cell. Signal.* 15, 299–306.
- [21] Takahashi, N., Kawada, T., Goto, T., Yamamoto, T., Taimatsu, A., Matsui, N., Kimura, K., Saito, M., Hosokawa, M., Miyashita, K. and Fushiki, T. (2002) *FEBS Lett.* 514, 315–322.
- [22] Wright, H.M., Clish, C.B., Mikami, T., Hauser, S., Yanagi, K., Hiramatsu, R., Serhan, C.N. and Spiegelman, B.M. (2000) *J. Biol. Chem.* 275, 1873–1877.
- [23] Gbaguidi, F.G., Chinetti, G., Milosavljevic, D., Teissier, E., Chapman, J., Olivecrona, G., Fruchart, J.C., Griglio, S., Fruchart-Najib, J. and Staels, B. (2002) *FEBS Lett.* 512, 85–90.
- [24] Chawla, A., Barak, Y., Nagy, L., Liao, D., Tontonoz, P. and Evans, R.M. (2001) *Nat. Med.* 7, 48–52.
- [25] Tontonoz, P., Hu, E., Graves, R.A., Budavari, A.I. and Spiegelman, B.M. (1994) *Genes Dev.* 8, 1224–1234.
- [26] Lundblad, J.R., Kwok, R.P., Lurance, M.E., Harter, M.L. and Goodman, R.H. (1995) *Nature* 374, 85–88.
- [27] Subbaramaiah, K., Lin, D.T., Hart, J.C. and Dannenberg, A.J. (2001) *J. Biol. Chem.* 276, 12440–12448.
- [28] Schluter, A., Barbera, M.J., Iglesias, R., Giralt, M. and Villarroya, F. (2002) *Biochem. J.* 362, 61–69.